Advanced Drug Delivery Reviews 135 (2018) 85–96

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Physiologically based pharmacokinetic modelling to guide drug delivery in older people

Manoranjenni Chetty [a,*], Trevor N. Johnson [a], Sebastian Polak [a,b], Farzaneh Salem [a], Kosuke Doki [c,d], Amin Rostami-Hodjegan [a,d]

[a] Simcyp Ltd (a Certara company), Blades Enterprise Centre, John Street, Sheffield, UK
[b] Unit of Pharmacoepidemiology and Pharmacoeconomics, Department of Social Pharmacy, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9 Str., 30-688 Cracow, Poland
[c] Department of Pharmaceutical Sciences, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan
[d] Centre for Applied Pharmacokinetic Research (CAPKR), University of Manchester, Manchester, UK

ARTICLE INFO

Article history:
Received 29 May 2017
Received in revised form 20 August 2018
Accepted 30 August 2018
Available online 4 September 2018

Keywords:
PBPK modelling in geriatrics
PBPK models informing drug delivery
Modelling drug delivery in older patients

ABSTRACT

Older patients are generally not included in Phase 1 clinical trials despite being the population group who use the largest number of prescription medicines. Physiologically based pharmacokinetic (PBPK) modelling provides an understanding of the absorption and disposition of drugs in older patients. In this review, PBPK models used for the prediction of absorption and exposure of drugs after parenteral, oral and transdermal administration are discussed. Comparisons between predicted drug pharmacokinetics (PK) and observed PK are presented to illustrate the accuracy of the predictions by the PBPK models and their potential use in informing clinical trial design and dosage adjustments in older patients. In addition, a case of PBPK modelling of a bioequivalence study on two controlled release products is described, where PBPK predictions reproduced the study showing bioequivalence in healthy volunteers but not in older subjects with achlorhydria, indicating further utility in prospectively identifying challenges in bioequivalence studies.

Contents

* Corresponding author at: Certara UK Limited, Simcyp Division, Level 2-Acero, 1 Concourse Way, Sheffield S1 2BJ, UK.
E-mail address: Manoranjenni.chetty@certara.com (M. Chetty).

https://doi.org/10.1016/j.addr.2018.08.013

1. Introduction

Despite being the population group that receive the largest number of drug prescriptions annually, older patients are generally not included in Phase 1 clinical trials during drug development. Complex pathophysiology, variability in organ function and the presence of co-medication are some of the factors that may account for the exclusion of older patients from clinical pharmacokinetic (PK) studies. Drug dose regimens used in the older patients are frequently determined by trial and error or extrapolated from doses relevant to young adults. Such extrapolations may not be appropriate since the scaling factors like age and weight that are used for extrapolation may not be linearly related to the PK variability associated with the older patient [1]. Considerable variability in drug response exists in the older patient [2,3], making dose selection very complex. In the absence of well-designed and appropriate clinical trials focused on the older patient, modelling and simulation offers a good tool to understand PK and pharmacodynamic (PD) variability and their impact on drug doses in this population.

Systems biology endeavours to quantify the contributions of the molecular elements of a biological system to assess their interactions and to integrate that information into models that can provide predictive hypotheses to explain the behaviours of the biological system when subjected to changes and different environmental exposures [4]. Systems pharmacology, a science pertinent to the study of drugs, has been described as a marriage between physiologically based pharmacokinetic (PBPK) modelling and *in vitro-in vivo* extrapolation (IVIVE) techniques under the umbrella of systems biology [5]. PBPK models encompass the principles of systems biology and constitute a modelling approach that describes the absorption and disposition of drug molecules as well as their biological and physiological drivers. An increased understanding of these physiological and biological drivers and their interactions with different drug molecules and formulations provide valuable insights into how drugs will behave in healthy volunteers and other population groups such as patients with diseases or extremes of age [6]. As illustrated in Fig. 1, based on the systems pharmacology approach, PBPK models constitute three essential components. These include biological and physiological factors relevant to the human body (systems related properties such as age, weight, sex, genetics, organ size and blood flow, enzyme abundance and activity, ethnicity etc.), drug related properties (e.g. physiochemical properties that determine permeability through membranes, protein binding, binding to enzymes and transporters etc.) and the study trial design (dose, frequency of dosing, duration of the study etc.). These components are combined and integrated mechanistically, enabling the simulation and prediction of PK/PD parameters and concentration-time and response-time profiles in virtual populations. Considering the progressive biological and physiological changes that accompany the process of aging, together with *co*-existing diseases and polypharmacy, IVIVE-linked PBPK models are ideally suited to predict the impact of these variables on the absorption and disposition of drugs in older patients. While PBPK modelling has the potential for mapping inter-individual variability in drug concentrations that reach the plasma following delivery by different

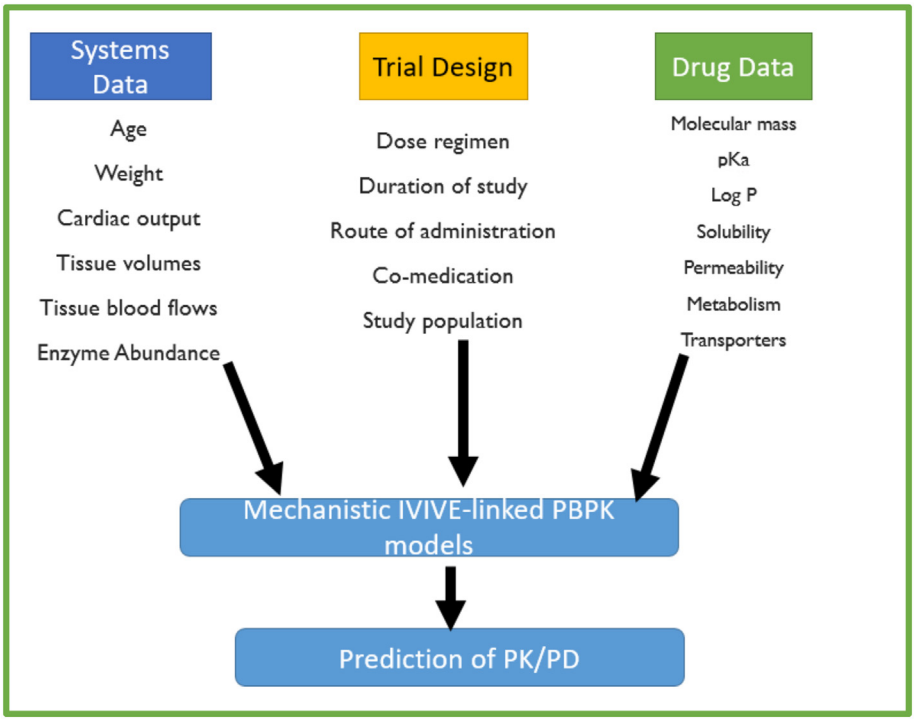

Fig. 1. Schematic representation of the systems pharmacology approach to population pharmacokinetic/pharmacodynamic modelling.

administration routes, it carries the added capability of predicting drug concentrations in the different organs in the body, which may be related to the site of action of the drug. A further advantage of using PBPK models is that 'what if' scenarios can be simulated to obtain information on the 'worst case' scenarios that cannot normally be tested in a clinical trial but may emerge after the drug is marketed.

Depending on the application, the structure of the PBPK model may be simple (minimal PBPK model) such as that shown in Fig. 2A (where all the tissues besides the liver and gut are lumped together as a single compartment) or more complex (whole body PBPK model), where all the major tissue compartments in the body are represented (Fig. 2B). Drug movement between the different tissues/organs are simulated using differential equations. Each organ compartment can be a simple static compartment with passive permeability or more mechanistic, where variables such as transporters, metabolizing enzymes and differential drug permeability are considered. Detailed descriptions of PBPK models, data requirements for effective PBPK models, differential equations used and applications in drug development and regulatory submissions have been extensively reviewed [5–12]. In this paper, applications of PBPK models to evaluate drug absorption, distribution, metabolism and excretion (ADME) in older subjects, following administration parenterally, orally and topically are presented. We also briefly discuss the role of formulation-related issues regarding variability and how they may interplay with age. The latter may be considered as re-visiting the wisdom of conducting bioequivalence studies in healthy volunteers rather than the patient groups for whom the drug product is intended, especially for drugs with properties such as pH dependent release and dissolution.

2. Overview of the methods used to predict PK parameters using IVIVE-linked PBPK models

This overview describes the PBPK models available in the Simcyp population based simulator. The brief summary should serve to illustrate how changes in the systems parameters can impact quantitatively on the PK of the drug.

2.1. Study Population

Default Simcyp parameter values required for creating a virtual North European Caucasian population (e.g. physiological parameters including liver volume and blood flows, enzyme abundances etc.) have been described previously [13]. Demographics, physiological and anatomical parameter values obtained from extensive literature searches are used to generate each study population, with variability between the subjects in the population. While the North European Caucasian population is the base population within the simulator, parameter changes relevant to other populations, such as Chinese or geriatrics, can be used to generate the special populations.

2.2. Prediction of PK

2.2.1. Absorption

Drug delivery from various routes of administration and different dosage forms can be used in the prediction of absorption and disposition of drugs. Oral absorption will be discussed briefly here since this is the most common route for drug delivery. Predictions can be made using a first-order absorption model, a compartmental absorption transit (CAT) model [14] or the advanced dissolution absorption metabolism (ADAM) model [11].

Bioavailability (F) of orally administered solid dosage forms involves release of the drug from the formulation, dissolution in the biological fluids, passage through the gut wall, and passage through the liver before it reaches the systemic circulation. Consequently, the following three parameters need to be considered: the fraction of the dose that enters the gut wall (fa), the fraction that escapes metabolism in the gut wall and enters the portal vein (F_G), and the fraction that enters the liver and escapes metabolism (F_H). The total bioavailability is the product of these three components (i.e., $F = fa \times F_G \times F_H$).

When the first-order absorption model is used, clinically observed inputs for the first order absorption rate constant (ka) and fraction absorbed (fa) can be used. Alternatively, these parameters can be predicted from an estimate of in vivo permeability, Peff,man, and the radius of the small intestine (R) [14], using Eqs. (1) and (2) below:

$$ka = \frac{2 \times P_{eff,man}}{R} \quad (1)$$

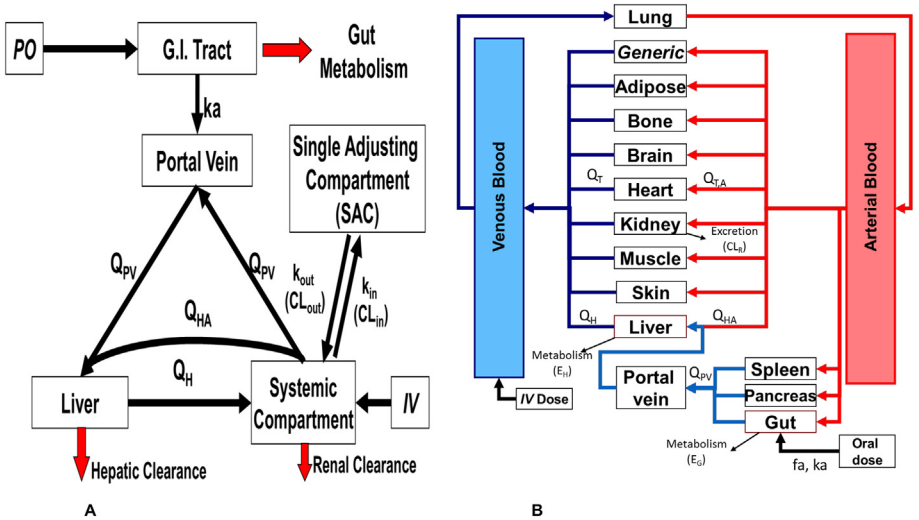

Fig. 2. A. Minimal physiologically-based pharmacokinetic model with (lower) single adjusting compartment. Q_H, Q_{PV}, and Q_{HA} are blood flows in the liver, portal vein, and hepatic artery, respectively; k_{in} and k_{out} are first order rate constants which act on the masses of drug within the systemic compartment and the SAC respectively, alternatively, the inter-compartmental clearance (Q) can be used; IV and PO are intravenous and oral dosing routes respectively; ka is the first order absorption rate constant. **B**. Whole body physiologically-based pharmacokinetic model. Q_H, Q_{HA}, Q_{PV}, Q_G, $Q_{T,A}$ and Q_T are blood flows in the hepatic vein, hepatic artery, hepatic portal vein, gut and blood flows into and out of the other tissue (T) compartments, respectively; E_G and E_H are the fractions undergoing first pass metabolism in the gut and liver, respectively; CL_R is the renal clearance; fa and ka are the fraction absorbed and the first order absorption rate constant, respectively.

$$fa = 1-(1 + 0.54\,P_{eff,man})^{-7} \quad (2)$$

The fraction of drug that escapes first pass metabolism and enters the portal vein (F_G) is influenced by the presence of enzymes and transporters in the gut [15]. F_G can be estimated using Eq. (3) [16], where Q_{gut} is a blood flow parameter, fu_{gut} is the unbound drug fraction in the gut and $CLu_{G,int}$ represents the gut intrinsic clearance:

$$F_G = \frac{Q_{gut}}{Q_{gut} + fu_{gut} \times CLu_{G,\,int}} \quad (3)$$

The fraction avoiding first-pass metabolism in the liver (F_H), can be calculated using Eq. (4), where Q_H (hepatic blood flow), fu_B (the fraction of drug unbound in blood) and $Clu_{int,H}$ (intrinsic metabolic clearance) are the primary determinants of net hepatic clearance (CL_H):

$$F_H = \frac{Q_H}{Q_H + fu_B.CLu_{\,int},H} \quad (4)$$

Fig. 3A illustrates a typical PBPK model for absorption that accounts for the differences in enzyme abundance (e.g. CYP3A4) and transporter abundance (e.g. P-glycoprotein) along the gastrointestinal tract. The model also accounts for other differences such as pH along the different segments. Fig. 3B represents a section of the small intestine, illustrating all the processes that can be simulated using ADAM [17].

2.2.2. Distribution

Factors such as tissue volumes and plasma:tissue partition coefficients are considered when predicting the volume of distribution of a drug. The following equation is used to predict V_{ss} when using the minimal PBPK model:

$$V_{ss} = \left(\sum V_t \times P_{t:p}\right) + (V_e \times E:P) + V_p \quad (5)$$

Where V is the fractional body volume (L/kg) of a tissue (t), erythrocyte (e), or plasma (p), E:P is the erythrocyte:plasma ratio and $P_{t:p}$ is the partition coefficient for non-adipose and adipose components [18].

2.2.3. Elimination

Metabolic drug elimination is defined in many physiologically based models using input data on intrinsic clearance (CL_{int}). This is scaled up based on specific enzyme abundance (CYP in case below), a scaling factor depending on which system is used (e.g. microsomes (MPPGL), hepatocytes etc.) and organ size (usually liver but could be kidney) to give an intrinsic clearance for the whole organ (Eq. (6)).

$$CL_{int} = \left[\sum_{j=1}^{n}(CL_{\,intj} \times CYP_j abundance)\right] \times MPPGL \times Liver\ Weight \quad (6)$$

The whole organ intrinsic clearance is then scaled up using a suitable model, usually the 'well stirred model' incorporating data on fraction of drug unbound in blood (fu_B), the whole organ intrinsic clearance (CL_{int}) values from all enzyme pathways and organ blood flow (Q_H) (Eq. (7)). The overall metabolic clearance in each organ is then added to the renal, biliary and any additional systemic clearance values to give the overall elimination of the drug.

$$CL_H = \frac{Q_H \times fu_B \times CLu_{int}}{Q_H + (fu_B \times CLu_{int})} \quad (7)$$

3. PBPK modelling in geriatric populations

PBPK models covering the geriatric population have been published previously [19,20], and were reported to predict PK parameters for a variety of drugs used in the elderly with reasonable accuracy. Many of the systems parameters, which form an important part of these models, change with age. A brief summary of the main changes likely to give rise to altered drug disposition in the older population is given below. These are included as part of the Simcyp PBPK Geriatric population and it is the development of this model that we concentrate on below. The database of Thompson *et al.* can be used as a starting point for establishing PBPK for the geriatric population which included those >65 years [21]. The review below mainly focusses on the major determinants of the factors governing drug elimination with mention of other areas relevant to building PBPK models in the geriatric population. For all parameters, only the changes resulting from the normal aging process are included. Whilst it is acknowledged that older patients often develop disease, those with overt renal or hepatic impairment are included in separate PBPK populations (moderate/severe renal impairment or liver cirrhosis Child-Pugh A, B, C) discussed previously [22,23].

3.1. Demographics

The typical age distribution seen in the elderly population is shown in Fig. 4A. This can be incorporated into PBPK using a Weibull function.

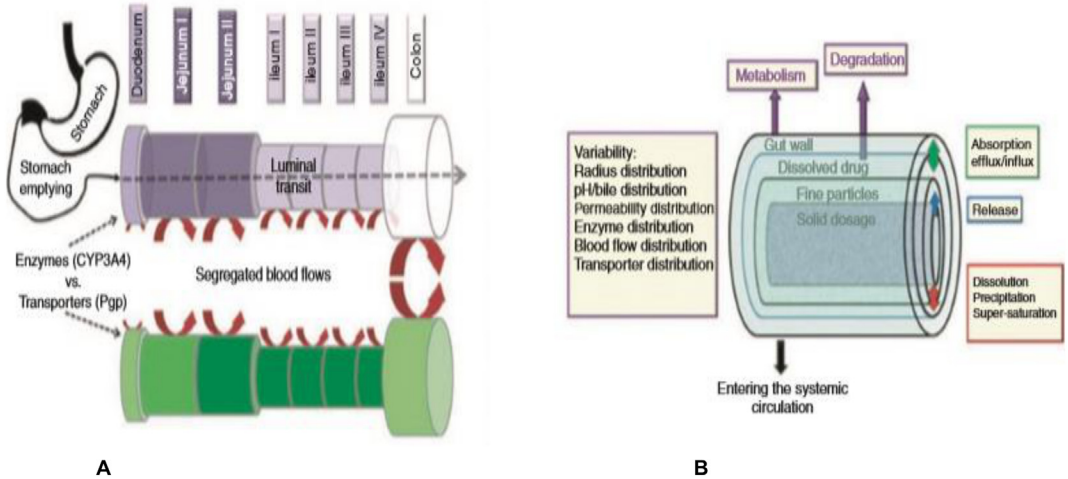

Fig. 3. A. Structure of the ADAM model in which the GI tract is divided into 9 sections with segregated blood flows to each section. The abundance of various enzymes and transporters in each segment varies non-monotonically along the intestine as indicated by the varying intensity of the colour for each section (CYP3A distribution is indicated in purple and P-gp in green). [17]. **B**. Segment of the small intestine indicating the various processes that can be simulated. [17].

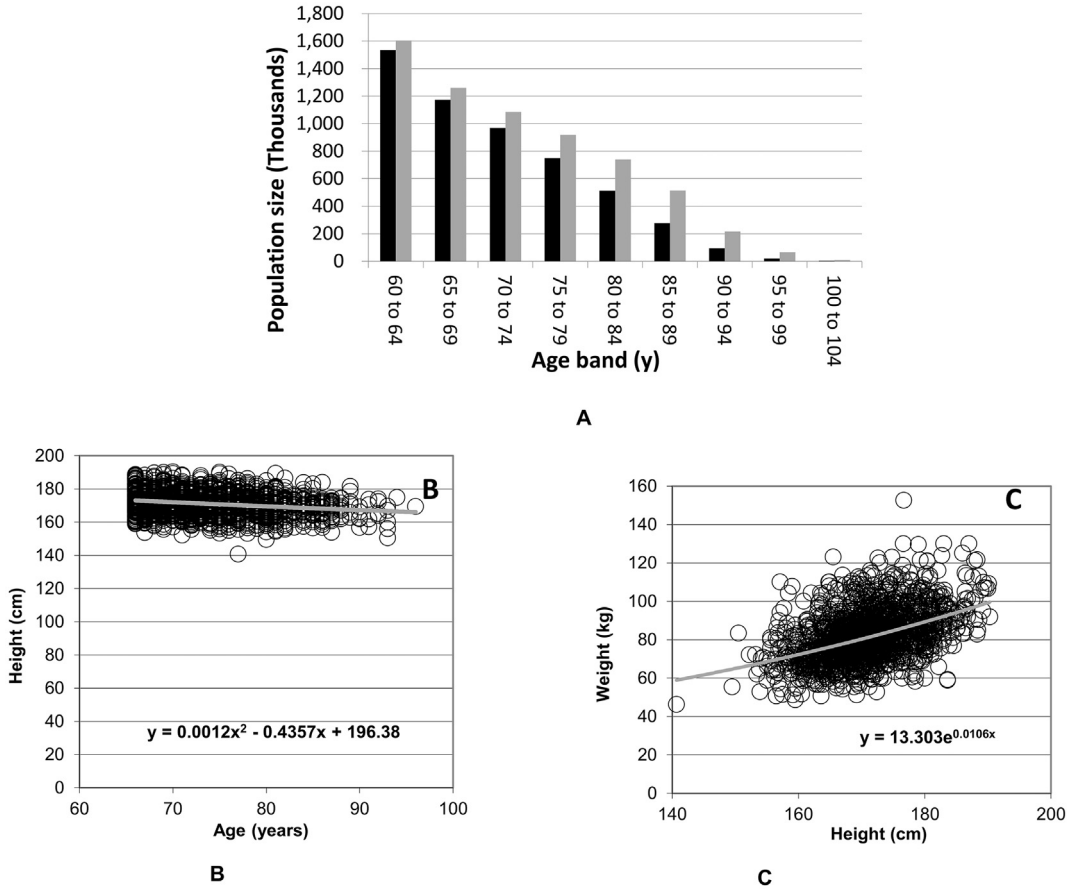

Fig. 4. A. The age distribution of a North European Geriatric population. Black and grey bars are males and females, respectively. **B**. The height vs age relationship for males, the black symbols are individual patient data from Health Survey for England data 2009 and the grey lines are best fit regression lines utilized in the geriatric PBPK model. **C**. The weight vs height relationship for males, the black symbols are individual patient data from Health Survey for England data 2009 and the grey lines are best fit regression lines utilized in the geriatric PBPK model.

There is a trend for reduced height with age with the rate of reduction in height increased in older age bands [20]. This is shown Fig. 4B based on Health survey for the UK [24]. To ensure a correlated Monte-Carlo approach body weight should be modelled based on height (Fig. 4C). This prevents the simulation of physiologically implausible virtual subjects. Similar to height, body weight also tends to decline in the older population with more rapid decline in older age bands.

3.2. Absorption

For oral absorption there is conflicting evidence on changes in gastric emptying (GE) and intestinal transit times (ITT) in the elderly, with studies showing both longer and shorter GE and ITT in this age group compared to a young adult group. Consequently, this parameter was left unchanged in the geriatric PBPK population [25,26]. There is evidence of a 10% increase in the Inter digestive Migrating Motor Complex in subjects in their eight decade compared to young adults. Weighted average was 112.4 min in 82 year olds ($n = 25$) compared to 102 min in 31 year olds ($n = 29$) [27,28]. This could impact on the biliary excretion of drugs and subsequently enterohepatic recirculation in the elderly.

3.3. Distribution

There are many organ systems where there is a decline in organ size with age and this has been reviewed in detail by Schlender et al. 2016 [20]. Within a PBPK model, changes to organ blood flows are related to the decline in cardiac output with age [29–31]. This is shown for cardiac output in males in Fig. 5A with simulated data compared to observed data.

3.4. Drug elimination

The main changes to the processes that may alter drug elimination with age and that will need to be incorporated into any geriatric PBPK model are given in the individual sections below.

3.4.1. Metabolism

Various studies have investigated age related changes in CYP enzymes with some conflicting results. Blanco et al. [32] investigated age related changes in microsomal CYP1A2 (ethoxyresorufin), CYP2E1 (ethoxycoumarin), CYP3A4/5 (midazolam) and CYP2C8 (paclitaxel 17α position) and found no age related change in activity between subjects >60 years compared to those >10 to 59 years. Previously Hunt et al. had also demonstrated no change in CYP3A activity in vitro with age (range 27 to 83 years) [33] and also in vivo using the erythromycin breath test between an older (70 to 88 years) compared to a younger age group (20 to 60 years) [34]. In another study the same authors showed no change in CYP2E1 activity over the range of 30 to 75 years [35]. Schmucker et al also demonstrated the absence of significant age differences in the activities and content of human liver monooxygenases [36]. In contrast George *et al* had shown a negative association between expression of CYP2E1 and CYP3A and older age [37].

A study by Parkinson *et al* 2004 investigated the effects of age on CYP activity in human liver microsomes [38]. Data was arranged into three age groups <20 y, 20 to 60 y and >60 y, each age group was composed of a minimum of 19 samples. Only CYPs 1A2, 2D6 and 2E1 were found

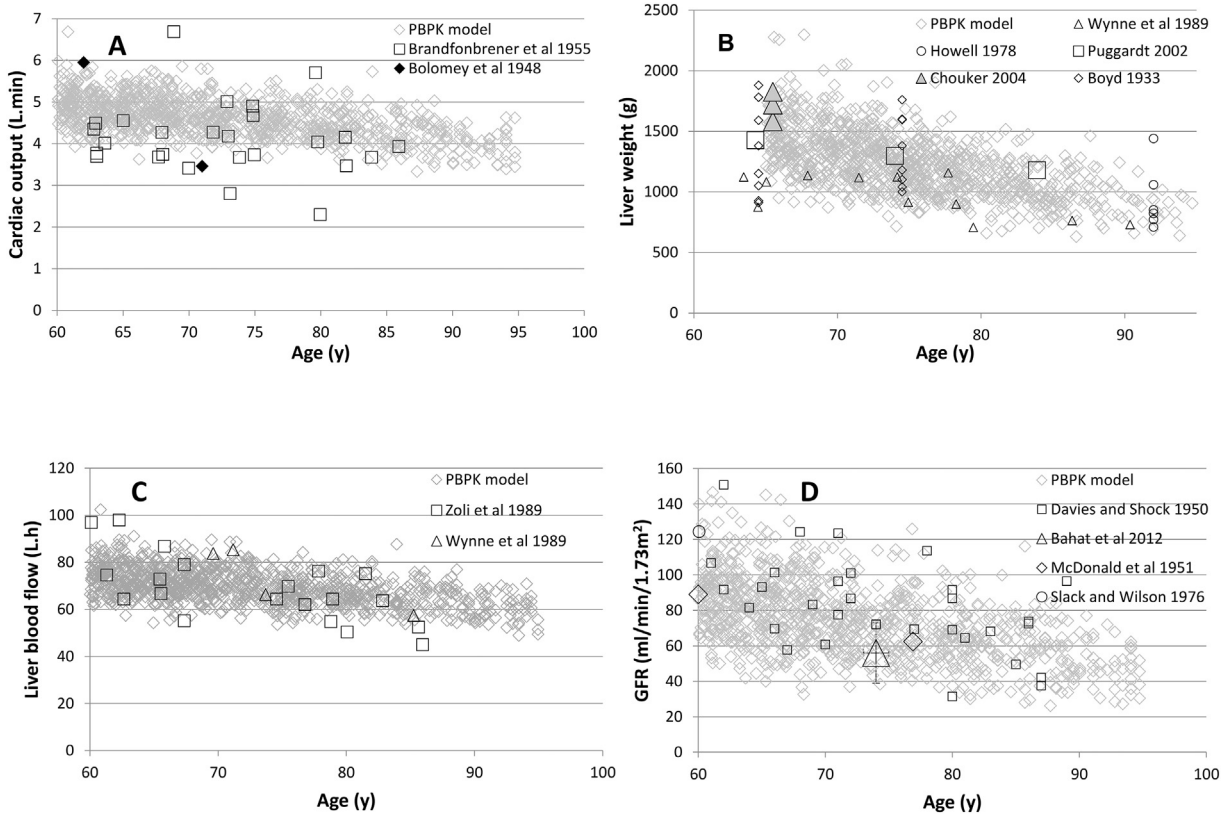

Fig. 5. Example 'Systems' data against age used in the geriatric PBPK model showing values generated by the model against measured values from the literature. Cardiac output for males is shown in **A**, liver weight for females is shown in **B,** liver blood flow for both males and females in **C** and GFR for males is shown in **D**. Grey symbols are model generated values and various black symbols are measured values (see references).

to be statistically significantly different with older age based on linear regression analysis, however, CYPs 1A2, 2B6, 2C19, 2D6 and 2E1 appeared to decrease >25% whilst CYPs 2A6 and 4A11 appeared to increase >25% between the oldest and youngest groups.

A study of in vivo drug metabolism based around administration of probe drug substrates to children and adolescents <19 years, adults 20 to 64 years and elderly adults >64 years [39] showed a decrease in metabolism of CYP1A2 (caffeine, theophylline), CYP2C9 tolbutamide, phenytoin and ibuprofen), CYP2C19 (amitriptyline and nortriptyline), CYP2E1 (acetaminophen) and CYP3A4 (midazolam, lidocaine and terfenadine) with increasing age. Whilst the suitability of a number of these probe drugs is questionable, a clear trend was observed but in light of other results these observations may have more to do with reducing liver size and blood flow rather than changes in specific enzyme expression and activity. In the final PBPK model no changes were implemented to the expression of CYP enzymes in the geriatric population and the same values as per the North European Caucasian population were used.

Older age is thought to have only a modest, if any, effect on hepatic glucuronidation of certain drugs in vivo [40], this is in agreement with *in vitro* data from a number of studies. Herd *et al.* [41] studied paracetamol glucuronidation (later confirmed as UGTs1A1, 1A9 and 2B15 [42]) activity in microsomes prepared from livers from individuals aged 44 to 89 years, and there was no significant correlation with age. Parkinson *et al.* [36] measured UGT activity in hepatocytes prepared from those aged 20 to 60 and those aged >60 years using 4-methylumbelliferone as a probe and found no difference between the groups. Further to this, Court *et al.* [43] studied age related changes in UGT activity in liver microsomes prepared from 55 individuals aged 5 to 75 years, taking out the paediatric liver samples this left data from 47 individuals >20 years and of these 13 who were >60 years. Using probe substrates, estradiol (UGT1A1), trifluoperazine (UGT1A4), serotonin (UGT1A6), propofol (UGT1A9), zidovudine (UGT2B7) and oxazepam (UGT2B15), again no age related changes in activity were observed between donors >60 and those <60 years. There is no evidence that the expression of UGT enzymes should be altered for the geriatric population in a PBPK model.

Little data exists for effects of older age on other drug metabolizing enzymes, the study by Herd *et al.* [41] additionally measured paracetamol sulphation from the cytosolic fractions in subjects 40 to 89 years and, similar to glucuronidation, found no correlation with age. A study by Farah *et al.* [44] measured the rate of acetylation of isoniazid in young adults (20 to 35 years) vs. the elderly (>65 years) and found no difference between the two populations. Finally Woodhouse *et al.* [45] measured both hepatic glutathione concentration and epoxide hydrolase activity and found no change with age.

3.4.2. Liver size

There is good evidence that liver size decreases with increasing age in the adult population with the decrease becoming more pronounced after 60 years of age [46–51], this data is summarized for females in Fig. 5B. The reduced effective liver mass results in a reduced capacity for drug elimination (Eq. (6)). This is matched by biopsy data showing a reduced number of hepatocytes in livers from elderly subjects [34]. The reduced liver size is incorporated into the geriatric population PBPK model.

3.4.3. Liver blood flow

The liver blood flow is defined in the geriatric PBPK model as a fixed percentage of cardiac output and hence is reduced with age, this is in agreement with observed data [52,53]. The simulated liver blood flow for males and females with some measured data are shown in Fig. 5C. In a study by Wynne *et al.* there was a significant negative correlation between indocyamine green clearance and apparent liver blood flow

and age [52]. The reduction in liver blood flow may result in reduced clearance for particularly intermediate and high extraction drugs in the elderly. Castleden *et al.* [54] showed a reduced systemic clearance of propranolol in older subject (mean age 78 years) compared to a younger group (mean age 29 years), they also showed higher bioavailability in the elderly due to reduced first-pass metabolism.

3.4.4. Protein binding

There appear to be very little change in plasma albumin concentrations with older age based on observed data [55–57] this is in agreement with the algorithms already used in the geriatric PBPK model which calculates albumin based on BMI and age. The observed data for older age related changes in alpha-1-acid glycoprotein show either no change [57] or a slight reduction with older age [56], no change with age for α-1 acid glycoprotein is currently included in the geriatric population PBPK model.

3.4.5. Renal elimination

The age related decline in renal function is well known [58–61]. The age related changes in glomerular filtration rate (GFR) in geriatric PBPK model are calculated using age related plasma creatinine data derived from the NHANES database [62] using either the Cockcroft-Gault or modified diet in Renal disease equations. The simulated and observed changes in GFR in older age are shown in Fig. 5D.

The magnitude of the changes in system parameters in the geriatric compared to HV populations within a PBPK model are shown in Table 1. The effects of these changes on the degree of change in the PK of actual and theoretical drugs of different extraction ratio has been predicted by the model and the results are in line with what is expected.

3.5. Prediction of clearance and exposure to six drugs in virtual geriatric patients following oral or parenteral administration

Clinical data on the PK of caffeine [64], desipramine [65], midazolam [66], digoxin [67], warfarin [19], triazolam [68] and omeprazole [69] in older patients was extracted from the literature. These compounds were available in the Simcyp compound library and literature data comparing the PK in younger and older subjects were available for comparison with predicted data. Initially, availability of clinical data that compares PK in young and older subjects is essential for performance verification of the PBPK models. The Geriatrics model described above was used to simulate the published studies and the predicted clearance and AUC was compared with the clinically observed values. Fig. 6A shows the predicted clearance values relative to the observed clearance and it can be seen that there is acceptable recovery of the observed data by the model. Fig. 6B shows the ratio of the predicted and observed % decrease in clearance between young and elderly patients. All these ratios are ≤1.5 (Fig. 6B) suggesting good recovery of the comparative clinical data between young and elderly subjects. While plasma concentrations in the older subjects were generally higher, some drug concentrations, such as those for midazolam, suggest that lower dosages may be more appropriate in this patient group.

Schlender and co-workers [20] used parenterally administered morphine and furosemide to verify a geriatrics model that they had developed. Satisfactory recovery of the clinical data was reported. These results indicate that both the Geriatrics models were successful in the prediction of exposure of these drugs following oral and parenteral delivery. Following verification of PBPK models, they can be used prospectively to predict differences in drug exposure between young and elderly subjects from different dosage formulations. Such predictions can assist with dosage adjustments in the elderly.

Table 1
Summary of changes to system parameters within a geriatric PBPK model resulting in altered ADME and thus predicted PK compared to a healthy volunteer population.

ADME	System parameter	Change compared to healthy volunteer population (Literature)	Changes to system parameters; geriatric PBPK model (65 to 95y) compared to Healthy volunteer (20 to 50 y)	Changes to system parameters with age in geriatric PBPK model (65 compared to 95 y)	Likely significance on predicted PK in elderly via PBPK model	References
Absorption	Gastric emptying	↑↓	None	–	V low	[25,26]
	Intestinal transit time	↑↓	None	–	V low	[25,26]
	Inter-digestive motor complex	↔↑	None	–	V low	[27,28]
Distribution	Organ size	↓	↓ (see below for liver)	–	V low (except liver)	[20]
	Cardiac output (organ blood flows)	↓	~18% ↓[e]	~16% ↓	Low to Moderate (see liver blood flow)	[52,53]
	Albumin	↔	~2% ↓[f]	~3% ↓	None	[55–57]
	α1-acid glycoprotein	↓↔	None	–	None	[56,57]
Metabolism	Liver weight	↓	~21% ↓[g]	~35% ↓	Moderate to high e.g. Midazolam[a] CL ↓32%,	[46–51]
	MPPGL	↔	~28% ↓[e]	~3% ↓	Midazolam with 10% CLint[b] then CL ↓40% in geriatrics vs HV	[63]
	Liver blood flow	See Cardiac output			Moderate for high extraction drugs e.g. Dextromethorphan[c] CL ↓22% in geriatric vs HV	
	CYP	↔↓	None		Low/Moderate	See text
	UGT	↔↓	None		Low/Moderate	See text
Elimination	Renal via GFR	↓	~47% ↓[h]	~45% ↓	Moderate to high e.g. Vancomycin[d] CL ↓47% in geriatric vs HV	[58–62]

[a] Intermediate extraction drug.
[b] Low extraction drug.
[c] High extraction drug.
[d] Renally cleared drug.
[e] Changes described in PBPK model using an age related equation in PBPK model.
[f] Changes described in PBPK model using an age and BSA related equation in PBPK model.
[g] Changes described in PBPK model using a BSA related equation up to 65y then an additional age related equation.
[h] Changes described in PBPK model via age related changes in serum creatinine.

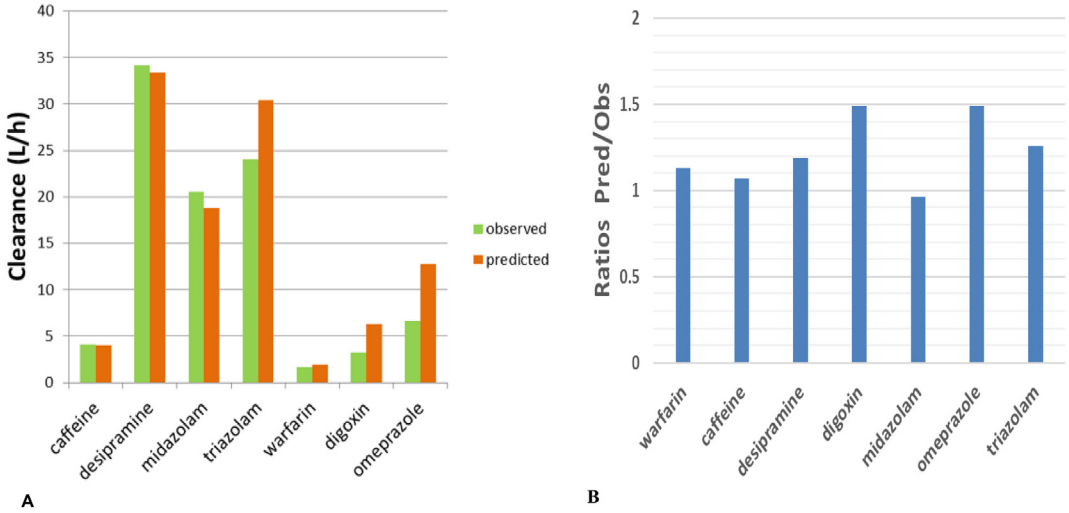

Fig. 6. A. Predicted clearance values relative to the observed clearance in geriatric patients. **B**. Ratios of the predicted and observed % decrease in clearance between young and elderly patients.

4. PBPK model for topical application

PBPK models are also useful tools allowing for prediction of systemic exposure following dermal administration. Combining information on genetic, physiological and demographic variability with preclinical *in vitro* data to allow extrapolation to *in vivo* population pharmacokinetics, the development of databases and models for dermal absorption can be considered as major advancement in the area of pharmaceutical science and drug delivery. A mechanistic dermal absorption model informed by human physiology (e.g. skin layer thickness, lipid contents, blood flow rates, etc.) was developed and integrated into the Simcyp Simulator to predict human dermal absorption of drugs [70]. This model was validated using 5 different diclofenac formulations. The effect of penetration enhancers, site of application, gender and ethnic variations were also incorporated in the simulated clinical trials. Good recovery of the observed drug exposure and population variability was evident in all five validation studies.

An enhanced version of this model is a transient, multi-phase and multi-layer (MPML) mechanistic dermal absorption model (MechDermA). The MPML MechDermA model (as shown in Fig. 7) is divided into several components: (1) formulation, (2) separate layers - stratum corneum, viable epidermis, dermis, subcutaneous tissue, muscle, (3) hair follicle, and (4) local vasculature (blood circulation). The model accounts for longitudinal diffusion and distribution processes

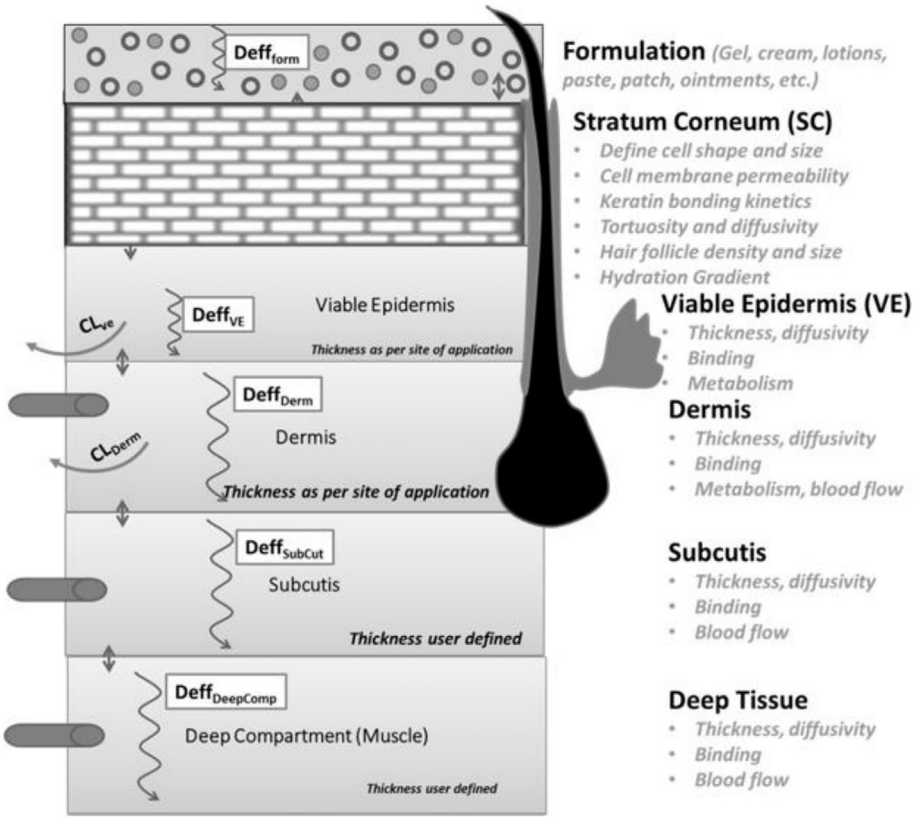

Fig. 7. Structure of the MechDermA mechanistic multi layer dermal absorption model showing the variables that may impact on absorption through each layer of the skin.

considering skin physiology related parameters (i.e. tortuosity of the diffusion pathway, keratin adsorption kinetics, stratum corneum (SC) hydration state, hair follicular transport, pH at the skin surface and within the SC layers, etc.) and drug/formulation specific parameters (i.e. ionization at the skin surface, lipophilicity, vehicle viscosity, etc.). The model accounts for variability in dermal physiology to simulate within-subject (multiple locations available) and between-subject variability, including the sex and age effect. Additionally MPML differentiates between various formulations beginning from simple solutions, through emulsions, pastes, and patches. The input parameters include *in vitro* measured formulation-specific information (i.e. *in vitro* release, number of micelles, viscosity etc.). Regardless of the formulation studied evaporation of the solvent can also be accounted for.

Age related changes to the physiology influence the ADME processes, and this includes disposition of drugs and other xenobiotics applied topically. The most commonly measured physiological properties of skin that influence the skin absorption are pH, skin and subcutaneous layers thickness and composition (amount and type of proteins and lipids as well as water content and their mutual ratios), local blood flow and the number of hair follicles. The MPML MechDermA PBPK model can be transformed into a model for dermal absorption in the older subjects based on parameter changes described below:

4.1. Skin surface pH

A meta-analysis of the skin surface pH data shows that in the volar forearm, neck and forehead there is a slight increase in this parameter in the elderly. However, measurement of pH at different areas of the back show a slight decrease in pH in elderly [71–78]. This could influence the absorption rate in the elderly, based on the site of application of the drug formulation.

4.2. Skin thickness

Escoffier and co-workers reported that skin maintains its total thickness up to about 65 years and becomes thinner thereafter [79]. However, for predicting dermal absorption of compounds it is necessary to understand thickness of skin layers as summarized in Table 2.

4.3. Blood flow

Comparison of adult and elderly blood flows shows no age related differences [76,102,103].

4.4. Hair follicles

Sinclair and coworkers presented a linear regression with scalp hair follicles and age in subjects between 13 and 84 years old [104]. To our knowledge there are no reports that compare the number of hair follicles in different body sites between adults and elderly.

All the above mentioned parameters can influence the drug dermatopharmacokinetics [105]. Roskos and colleagues presented results of the study comparing permeation of various compounds: in the elderly compared with young individual [106]. According to their observations hydrophilic compounds (hydrocortisone, benzoic acid, acetylsalicylic acid, and caffeine) absorption was significantly lower, whereas lipophilic drugs behaviour (testosterone and estradiol) was not affected by age. Holdsworth et al. compared fentanyl disposition after a 24-h application of fentanyl transdermal patch in 10 healthy elderly subjects 67–87 years and 6 young subjects 19–27 years [107]. The observed higher serum concentrations reflect increased absorption and/or decreased clearance in the elderly.

Table 2
Summary of changes in skin layers applicable to the MPML Geriatrics model.

Skin layer	MPML geriatrics model	Comment	References
Stratum Corneum (SC) thickness		Based on corneocyte hydration, corneocyte dimensions and the number of dead cell layers in SC	
Corneocyte surface area	Mean ratio of increase from adult to elderly applied to model: Cheek 1.2 Upper arm 1.3 Volar forearm 1.2 Dorsal forearm 1.7 Thigh 1.1 Forehead 1.2	Age-related increase in surface area of corneocytes	[71,81,82]
Number of SC layers	No change in the number of layers between young adults and elderly included in the model	Small studies suggested increase Large studies showed no change	[77,83,84] [79,80,85]
Corneocyte hydration	No significant change reported between young adults and elderly	Capacitance was used as a measure of hydration	[86,87,88]
Thickness of the viable epidermis	No significant change between young adults and elderly	Reported ratios indicate negligible changes	[79,80,83,89,90]
Thickness of dermis	Minor decreases in the elderly in some areas: Upper arm 0.7 Cheek 0.9	Based on a meta-analysis	[70,90,91]
Thickness of subcutaneous tissue	Elderly Upper arm 11 mm Back 6.4 mm	Limited data available	[92,93,94]
Muscle thickness	Elderly Lower leg: Males 16.4 mm Females 17.4 mm Upper leg: Males 27.6 mm Females 22.2 mm Upper arm: Males 22.7 mm Females 16.5 mm	Age-dependent decline in thickness is faster in males	[95,96,97-101]

5. An example of interplay between age, ethnicity and formulation leading to variable exposure

Bioequivalence studies are typically conducted in healthy volunteers. There has been a continuous discussion and debate on the necessity of conducting bioequivalence studies in the target population of patients (most of them being of older age). There are a variety of reasons, as outlined above, which makes the kinetics of the drug different in elderly populations. However, most of these would affect the drug regardless of the formulation. Hence, they may not be expected to have material impact on bioequivalence. The only exception is the impact of formulation during the absorption phase (whether oral or non-oral). The components of the formulation may introduce sensitivities to age-related attributes of the gastro-intestinal tract which leads to disparity between the drug products performance when they are compared with what happens in a healthy volunteer population. We describe such potential interplay combined with the effect of ethnic influence based on a recent report.

The case involves one formulation being influenced by achlorhydria to a much greater extent than the other. In a simulated comparison of the test and reference nifedipine controlled release formulations, the two formulations were shown to be bioequivalent in healthy volunteers (as observed in the clinical trial) but not in older Japanese subjects who have achlorhydria. Achlorhydric patients show elevated gastric pH which may lead to differential effects on various products of the same drug based on pH-sensitivity of the formulation. Doki et al. [108] examined the latter via PBPK simulations involving the mechanistic oral absorption model ADAM. The virtual bioequivalence (V-BE) studies highlighted the need for conduct of specific studies in elderly Japanese populations where there are discrepancies in pH-sensitivity of dissolution between the test and reference formulations.

6. Conclusions

The above discussions illustrate the utility of the application of PBPK models to predict the drug exposure resulting from different formulations and different routes of administration. Prediction of the differences in systemic drug exposure between young adults and older subjects can be used to inform clinical trial designs and highlight situations where dosage adjustments for the older subjects require consideration. PBPK models are not a substitute for the conduct of clinical studies in this population. However, they can highlight the possibility for discrepant bioequivalence or drug PK from those established in healthy volunteers and guide the conduct of clinical studies when there is adequate evidence for a likely difference. This approach may inform clinical testing during drug development, thus avoiding the conduct of logistically more difficult studies in the elderly in cases where they may not be needed.

Conflict of interest/declaration

M. Chetty, TN Johnson, S.Polak and F Salem are employees of Simcyp Limited (a Certara company). A. Rostami-Hodjegan is an employee of the University of Manchester and part-time secondee to Simcyp Limited (a Certara Company).

Author contributions

All the authors contributed to the research and the writing of the manuscript.

References

[1] C. Johnston, C.M. Kirkpatrick, A.J. McLachlan, S.N. Hilmer, Physiologically based pharmacokinetic modeling at the extremes of age, Clin. Pharmacol. Ther. 93 (2013) 148.
[2] A.J. McLachlan, S.N. Hilmer, D.G. Le Couteur, Variability in response to medicines in older people: phenotypic and genotypic factors, Clin. Pharmacol. Ther. 85 (2009) 431–433.
[3] S.N. Hilmer, A.J. McLachlan, D.G. Le Couteur, Clinical pharmacology in geriatric patients, Fundam. Clin. Pharmacol. 21 (2007) 217–230.
[4] L. Hood, J.R. Heath, M.E. Phelps, B. Lin, Systems Biology and New Technologies Enable Predictive and Preventative Medicine, Science 306 (2004) 640–643.
[5] A. Rostami-Hodjegan, Physiologically based pharmacokinetics joined with *in vitro–in vivo* extrapolation of ADME: a marriage under the arch of systems pharmacology, Clin. Pharmacol. Ther. 92 (2012) 50–61.
[6] M. Rowland, L.J. Lesko, A. Rostami-Hodjegan, Physiologically based pharmacokinetics is impacting drug development and regulatory decision making, CPT Pharmacometrics Syst. Pharmacol. 4 (2015) 313–315.
[7] A. Rostami-Hodjegan, G.T. Tucker, Simulation and prediction of in vivo drug metabolism in human populations from in vitro data, Nat. Rev. Drug Discov. 6 (2007) 140–148.
[8] M. Jamei, G. Dickinson, A. Rostami-Hodjegan, A framework for assessing interindividual variability in pharmacokinetics using virtual human populations and integrating general knowledge of physical chemistry,biology,anatomy, physiologyandgenetics: a tale of 'bottom-up' vs 'top-down' recognition of covariates, Drug Metab. Pharmacokinet. 24 (2009) 53–75.
[9] M. Jamei, S. Marciniak, D. Edwards, K. Wragg, K. Feng, A. Barnett, A. Rostami-Hodjegan, The simcyp population based simulator: architecture, implementation, and quality assurance, In Silico Pharmacol. 3 (2013) 1–9.
[10] J.E. Sager, J. Yu, I. Ragueneau-Majlessi, N. Isoherranen, Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications and model verification, Drug Metab. Dispos. 43 (2015) 1823–1837.
[11] M. Jamei, D. Turner, J. Yang, S. Neuhoff, S. Polak, A. Rostami-Hodjegan, G. Tucker, Population-based mechanistic prediction of oral drug absorption, AAPS J. 11 (2009) 225–237.
[12] S. Hansmann, A. Darwich, A. Margolskee, L. Aarons, J. Dressman, Forecasting oral absorption across biopharmaceutics classification system classes with physiologically based pharmacokinetic models, J. Pharm. Pharmacol. 68 (2016) 1501–1515.
[13] E.M. Howgate, K. Rowland-Yeo, N.J. Proctor, G.T. Tucker, A. Rostami-Hodjegan, Prediction of in vivo drug clearance from in vitro data: impact of interindividual variability, Xenobiotica 36 (2006) 473–497.
[14] L.X. Yu, G.L. Amidon, Saturable small intestine drug absorption in humans: modelling and interpretation of cefatrizine data, Eur. J. Pharm. Biopharm. 45 (1998) 199–203.
[15] K.S. Pang, Modeling of intestinal drug absorption: roles of transporters and metabolic enzymes (for the Gillette Review Series), Drug Metab. Dispos. 31 (2003) 1507–1519.
[16] A. Rostami-Hodjegan, G.T. Tucker, 'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions, Drug Discov. Today (2004) 441–448.
[17] A. S. Darwich, S. Neuhoff, M. Jamei, A. Rostami-Hodjegan, Interplay of metabolism and transport in determining oral drug absorption and gut wall metabolism: a simulation assessment using the "Advanced Dissolution, Absorption, Metabolism (ADAM)" model, Curr. Drug Metab. 11 (2010) 716–729.
[18] Y. Sawada, M. Hanano, Y. Sugiyama, H. Harashima, T. Iga, Prediction of the volumes of distribution of basic drugs in humans based on data from animals, J. Pharmacokinet. Biopharm. 12 (1984) 587–596.
[19] T.M. Polasek, F. Patel, B.P. Jensen, M.J. Sorich, M.D. Wiese, M.P. Doogue, Predicted metabolic drug clearance with increasing adult age, Br. J. Clin. Pharmacol. 75 (2013) 1019–1028.
[20] F. Schlender, M. Meyer, K. Thelen, M. Krauss, S. Willmann, T. Eissing, U. Jaehde, Development of a whole-body physiologically based pharmacokinetic approach to assess the pharmacokinetics of drugs in elderly individuals, Clin. Pharmacokinet. 55 (2016) 1573–1589.
[21] C.M. Thompson, D.O. Johns, B. Sonawane, H.A. Barton, D. Hattis, R. Tardif, K. Krishnan, Database for physiologically based pharmacokinetic (PBPK) modeling: physiological data for healthy and health-impaired elderly, J. Toxicol. Environ. Health B Crit. Rev. 12 (2009) 1–24.
[22] K. Rowland Yeo, M. Aarabi, M. Jamei, A. Rostami-Hodjega, Modeling and predicting drug pharmacokinetics in patients with renal impairment, Expert. Rev. Clin. Pharmacol. 4 (2011) 261–274.
[23] T.N. Johnson, K. Boussery, K. Rowland Yeo, G.T. Tucker, A. Rostami-Hodjegan, A semi-mechanistic model to predict the effects of liver cirrhosis on drug clearance, Clin. Pharmacokinet. 49 (2010) 189–206.
[24] Health survey for England, http://content.digital.nhs.uk/pubs/hse09report 2009.
[25] A. Brogna, R. Ferrara, A.M. Bucceri, E. Lanteri, F. Catalano, Influence of aging on gastrointestinal transit time. An ultrasonographic and radiologic study, Invest. Radiol. 34 (1999) 357–359.
[26] J.L. Madsen, J. Graff, Effects of ageing on gastrointestinal motor function, Age Ageing 33 (2004) 154–159.
[27] M. Bortolotiti, G. Frada, G. Barbagallo-Sangiorgi, G. Labo, Interdigestive gastroduodenal motor activity in the elderly, Gut 25 (1984) A1320.
[28] E. Husebye, K. Engedal, The patterns of motility are maintained in the human small intestine throughout the process of aging, Scand. J. Gastroenterol. 27 (1992) 397–404.
[29] A.A. Bolomey, A.J. Michie, et al., Simultaneous measurement of effective renal blood flow and cardiac output in resting normal subjects and patients with essential hypertension, J. Clin. Invest. 28 (1949) 10–17.
[30] M. Brandfonbrener, M. Landowne, N.W. Shock, Changes in cardiac output with age, Circulation 12 (1955) 557–566.

[31] A.A. Luisada, P.K. Bhat, V. Knighten, Changes of cardiac output caused by aging: an impedance cardiographic study, Angiology 31 (1980) 75–81.
[32] J.G. Blanco, P.L. Harrison, W.E. Evans, M.V. Relling, Human cytochrome P450 maximal activities in pediatric versus adult liver, Drug Metab. Dispos. 28 (2000) 379–382.
[33] C.M. Hunt, W.R. Westerkam, G.M. Stave, Effect of age and gender on the activity of human hepatic CYP3A, Biochem. Pharmacol. 44 (1992) 275–283.
[34] C.M. Hunt, W.R. Westerkam, G.M. Stave, J.A. Wilson, Hepatic cytochrome P-4503A (CYP3A) activity in the elderly, Mech. Ageing Dev. 64 (1992) 189–199.
[35] C.M. Hunt, S. Strater, G.M. Stave, Effect of normal aging on the activity of human hepatic cytochrome P450IIE1, Biochem. Pharmacol. 40 (1990) 1666–1669.
[36] D.L. Schmucker, Age-related changes in liver structure and function: Implications for disease? Exp. Gerontol. 40 (2005) 650–659.
[37] J. George, K. Byth, G.C. Farrell, Age but not gender selectively affects expression of individual cytochrome P450 proteins in human liver, Biochem. Pharmacol. 50 (1995) 727–730.
[38] A. Parkinson, D.R. Mudra, C. Johnson, A. Dwyer, K.M. Carroll, The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes, Toxicol. Appl. Pharmacol. 199 (2004) 193–209.
[39] E. Tanaka, In vivo age-related changes in hepatic drug-oxidizing capacity in humans, J. Clin. Pharm. Ther. 23 (1998) 247–255.
[40] D.J. Greenblatt, M. Divoll, J.S. Harmatz, R.I. Shader, Oxazepam kinetics: effects of age and sex, J. Pharmacol. Exp. Ther. 215 (1980) 86–91.
[41] B. Herd, H. Wynne, P. Wright, O. James, K. Woodhouse, The effect of age on glucuronidation and sulphation of paracetamol by human liver fractions, Br. J. Clin. Pharmacol. 32 (1991) 768–770.
[42] X.L. Jiang, P. Zhao, J.S. Barrett, L.J. Lesko, S. Schmidt, Application of physiologically based pharmacokinetic modeling to predict acetaminophen metabolism and pharmacokinetics in children, CPT Pharmacometrics Syst. Pharmacol. 2 (2013), e80. .
[43] M.H. Court, Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system, Drug Metab. Rev. 42 (2010) 209–224.
[44] F. Farah, W. Taylor, M.D. Rawlins, O. James, Hepatic drug acetylation and oxidation: effects of aging in man, Br. Med. J. 2 (1977) 155–156.
[45] K.W. Woodhouse, E. Mutch, F.M. Williams, M.D. Rawlins, O.F. James, The effect of age on pathways of drug metabolism in human liver, Age Ageing 13 (1984) 328–334.
[46] E. Boyd, Normal variability in weight of the adult human liver and spleen, Arch. Pathol. 16 (1933) 350–372.
[47] N.O. Calloway, C.F. Foley, P. Lagerbloom, Uncertainties in geriatric data. Ii. Organ size, J. Am. Geriatr. Soc. 13 (1965) 20–28.
[48] A. Chouker, A. Martignoni, M. Dugas, W. Eisenmenger, R. Schauer, I. Kaufmann, G. Schelling, F. Lohe, K.W. Jauch, K. Peter, M. Thiel, Estimation of liver size for liver transplantation: the impact of age and gender, Liver Transpl. 10 (2004) 678–685.
[49] T.H. Howell, Organ weights in nonagenarians, J. Am. Geriatr. Soc. 26 (1978) 385–390.
[50] L. Puggaard, K.S. Bjornsbo, K. Kock, K. Luders, B. Thobo-Carlsen, O. Lammert, Age-related decrease in energy expenditure at rest parallels reductions in mass of internal organs, Am. J. Hum. Biol. 14 (2002) 486–493.
[51] H.A. Wynne, L.H. Cope, E. Mutch, M.D. Rawlins, K.W. Woodhouse, O.F. James, The effect of age upon liver volume and apparent liver blood flow in healthy man, Hepatology 9 (1989) 297–301.
[52] H.A. Wynne, J. Goudevenos, M.D. Rawlins, O.F. James, P.C. Adams, K.W. Woodhouse, Hepatic drug clearance: the effect of age using indocyanine green as a model compound, Br. J. Clin. Pharmacol. 30 (1990) 634–637.
[53] M. Zoli, T. Iervese, S. Abbati, G.P. Bianchi, G. Marchesini, E. Pisi, Portal blood velocity and flow in aging man, Gerontology 35 (1989) 61–65.
[54] C.M. Castleden, C.F. George, The effect of ageing on the hepatic clearance of propranolol, Br. J. Clin. Pharmacol. 7 (1979) 49–54.
[55] E.W. Campion, L.O. Delabry, R.J. Glynn, The effect of age on serum albumin in healthy males: report from the Normative Aging Study, J. Gerontol. 43 (1988) M18–M20.
[56] B.T. Veering, A.G. Burm, J.H. Souverijn, J.M. Serree, J. Spierdijk, The effect of age on serum concentrations of albumin and alpha 1-acid glycoprotein, Br. J. Clin. Pharmacol. 29 (1990) 201–206.
[57] R.K. Verbeeck, J.A. Cardinal, S.M. Wallace, Effect of age and sex on the plasma binding of acidic and basic drugs, Eur. J. Clin. Pharmacol. 27 (1984) 91–97.
[58] G. Bahat, F. Tufan, S. Akin, T.S. Akpinar, A. Tufan, S. Ozturk, B. Saka, Y. Sanli, G. Tugrul, L. Adalet, B. Oner, N. Erten, M.A. Karan, Which method is better in the elderly to estimate glomerular filtration rate? Eur. Geriatr. Med. (3) (2012) S106.
[59] D.F. Davies, N.W. Shock, Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males, J. Clin. Invest. 29 (1950) 496–507.
[60] D.R. Mc, D.H. Solomon, N.W. Shock, Aging as a factor in the renal hemodynamic changes induced by a standardized pyrogen, J. Clin. Invest. 30 (1951) 457–462.
[61] T.K. Slack, D.M. Wilson, Normal renal function: CIN and CPAH in healthy donors before and after nephrectomy, Mayo Clin. Proc. 51 (1976) 296–300.
[62] N.C.f.H. Statistics, National Health and Nutrition Examination Survey, 2018 https://www.cdc.gov/nchs/nhanes/index.htm Accessed September 2018.
[63] Z.E. Barter, J.E. Chowdry, J.R. Harlow, J.E. Snawder, J.C. Lipscomb, A. Rostami-Hodjegan, Covariation of human microsomal protein per gram of liver with age: absence of influence of operator and sample storage may justify interlaboratory data pooling, Drug Metab. Dispos. 36 (2008) 2405–2409.
[64] J. Blanchard, S.J.A. Sawers, Comparative pharmacokinetics of caffeine in young and elderly men, J. Pharmacokinet. Biopharm. 11 (1983) 109–126.
[65] D.A. Abernethy, D.J. Greenblatt, R.I. Shader, Imipramine and desipramine disposition in the elderly, J. Pharm. Exp. Ther. 232 (1984) 183–188.
[66] H.-P. Platten, E. Schweizer, K. Dilger, G. Mikus, U. Klotz, Pharmacokinetics and the pharmacodynamic action of midazolam in young and elderly patients undergoing tooth extraction, Clin. Pharmacol. Ther. 63 (1998) 552–560.
[67] B. Cusack, J. Kelly, K. O'Malley, J. Noel, Digoxin in the elderly: pharmacokinetic consequences of old age, Clin. Pharmacol. Ther. 25 (1974) 772–776.
[68] D.J. Greenblatt, J.S. Harmatz, L. Shapiro, N. Engelhardt, T.A. Gouthro, R.I. Shader, Sensitivity to triazolam in the elderly, N. Engl. J. Med. 324 (1991) 1691–1698.
[69] S. Landahl, T. Andersson, M. Larsson, B. Lernfeldt, P. Lundborg, C.-G. Regardh, E. Six, I. Skanberg, Pharmacokinetic study of omeprazole in elderly healthy volunteers, Clin. Pharmacokinet. 23 (1992) 469–476.
[70] S. Polak, C. Ghobadi, H. Mishra, M. Ahamadi, N. Patel, M. Jamei, A. Rostami-Hodjegan, Prediction of concentration–time profile and its inter-individual variability following the dermal drug absorption, J. Pharm. Sci. 101 (2012) 2584–2595.
[71] S.H.-A. Dikstein, P. Bercovici, The dependance of low pressure indentation, slackness and surface pH on age in forehead skin of women, J. Soc. Cosmet. Chem. 35 (1984) 221–228.
[72] A. Zlotogorski, Distribution of skin surface pH on the forehead and cheek of adults, Arch. Dermatol. Res. 279 (1987) 398–401.
[73] P. Thune, T. Nilsen, I.K. Hanstad, T. Gustavsen, H. Lovig Dahl, The water barrier function of the skin in relation to the water content of stratum corneum, pH and skin lipids. The effect of alkaline soap and syndet on dry skin in elderly, non-atopic patients, Acta Derm. Venereol. 68 (1988) 277–283.
[74] K.P. Wilhelm, A.B. Cua, H.I. Maibach, Skin aging. Effect on transepidermal water loss, stratum corneum hydration, skin surface pH, and casual sebum content, Arch. Dermatol. 127 (1991) 1806–1809.
[75] G. Yosipovitch, E. Tur, O. Cohen, Y. Rusecki, Skin surface pH in intertriginous areas in NIDDM patients. Possible correlation to candidal intertrigo, Diabetes Care 16 (1993) 560–563.
[76] S. Marrakchi, H.I. Maibach, Biophysical parameters of skin: map of human face, regional, and age-related differences, Contact Dermatitis 57 (2007) 28–34.
[77] S. Schreml, V. Zeller, R.J. Meier, H.C. Korting, B. Behm, M. Landthaler, P. Babilas, Impact of age and body site on adult female skin surface pH, Dermatology 224 (2012) (2012) 66–71.
[78] S. Luebberding, N. Krueger, M. Kerscher, Age-related changes in skin barrier function - quantitative evaluation of 150 female subjects, Int. J. Cosmet. Sci. 35 (2013) 183–190.
[79] C. Escoffier, J. de Rigal, A. Rochefort, R. Vasselet, J.L. Leveque, P.G. Agache, Age-related mechanical properties of human skin: an in vivo study, J. Invest. Dermatol. 93 (1989) 353–357.
[80] E. Berardesca, Corneocyte Size and Cell Renewal: Effects of Aging and Sex Hormones, Textbook of Aging Skin, Springer, 1968.
[81] C. Blair, Morphology and thickness of the human stratum corneum, Br. J. Dermatol. 80 (1968) 430–436.
[82] D. Batisse, R. Bazin, T. Baldeweck, B. Querleux, J.L. Leveque, Influence of age on the wrinkling capacities of skin, Skin Res. Technol. 8 (2002) 148–154.
[83] M.J. Koehler, T. Vogel, P. Elsner, K. Konig, R. Buckle, M. Kaatz, In vivo measurement of the human epidermal thickness in different localizations by multiphoton laser tomography, Skin Res. Technol. 16 (2010) 259–264.
[84] G.L. Grove, Exfoliative cytological procedures as a nonintrusive method for dermatogerontological studies, J. Invest. Dermatol. 73 (1979) 67–69.
[85] N. Kashibuchi, Y. Hirai, K. O'Goshi, H. Tagami, Three-dimensional analyses of individual corneocytes with atomic force microscope: morphological changes related to age, location and to the pathologic skin conditions, Skin Res. Technol. 8 (2002) 203–211.
[86] K. Cucumel, B.J. Botto, N. Domloge, C. Dal Farra, in: T. Nagata (Ed.), Age-Related Changes in Human Skin by Confocal Laser Scanning Microscope. Senesence, 2012 https://doi.org/10.5772/5316 , Chapter 32.
[87] H. Baker, C.P. Blair, Cell replacement in the human stratum corneum in old age, Br. J. Dermatol. 80 (1968) 367–372.
[88] Z. Ya-Xian, T. Suetake, H. Tagami, Number of cell layers of the stratum corneum in normal skin - relationship to the anatomical location on the body, age, sex and physical parameters, Arch. Dermatol. Res. 291 (1999) 555–559.
[89] M. Egawa, T. Hirao, M. Takahashi, In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy, Acta Derm. Venereol. 87 (2007) 4–8.
[90] K.P. Wilhelm, A.B. Cua, H.I. Maibach, Skin aging. Effect on transepidermal water loss, stratum corneum hydration, skin surface pH, and casual sebum content, Arch. Dermatol. 127 (1991) 1806–1809.
[91] N. Sato, T. Kitahara, T. Fujimura, Age-related changes of stratum corneum functions of skin on the trunk and the limbs, Skin Pharmacol. Physiol. 27 (2014) 181.
[92] J. Sandby-Moller, T. Poulsen, H.C. Wulf, Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits, Acta Derm. Venereol. 83 (2003) 410–413.
[93] M.C. Branchet, S. Boisnic, C. Frances, A.M. Robert, Skin thickness changes in normal aging skin, Gerontology 36 (1990) (1990) 28–35.
[94] D. Crisan, M. Lupsor, M. Crisan, C. Napoca Cluj-Napoca, Skin Physiology and Skin Manifestations According to Age: An Ultrasonographic Approach, EPOS, 2012.
[95] J.S. Petrofsky, K. McLellan, G.S. Bains, M. Prowse, G. Ethiraju, S. Lee, S. Gunda, E. Lohman, E. Schwab, Skin heat dissipation: the influence of diabetes, skin thickness, and subcutaneous fat thickness, Diabetes Technol. Ther. 10 (2008) 487–493.
[96] M.A. Gibney, C.H. Arce, K.J. Byron, L.J. Hirsch, Skin and subcutaneous adipose layer thickness in adults with diabetes at sites used for insulin injections: implications for needle length recommendations, Curr. Med. Res. Opin. 26 (2010) 1519–1530.

[97] K. Hwang, H. Kim, D.J. Kim, Thickness of skin and subcutaneous tissue of the free flap donor sites: a histologic study, Microsurgery 36 (2016) 54–58.
[98] I.M. Arts, S. Pillen, H.J. Schelhaas, S. Overeem, M.J. Zwarts, Normal values for quantitative muscle ultrasonography in adults, Muscle Nerve 41 (2010) (2010) 32–41.
[99] K. Kubo, H. Kanehisa, K. Azuma, M. Ishizu, S.Y. Kuno, M. Okada, T. Fukunaga, Muscle architectural characteristics in young and elderly men and women, Int. J. Sports Med. 24 (2003) 125–130.
[100] T. Yoshimatsu, D. Yoshida, H. Shimada, T. Komatsu, A. Harada, T. Suzuki, Relationship between near-infrared spectroscopy, and subcutaneous fat and muscle thickness measured by ultrasonography in Japanese community-dwelling elderly, Geriatr Gerontol Int 13 (2013) (2013) 351–357.
[101] T. Binzoni, S. Bianchi, S. Hanquinet, A. Kaelin, Y. Sayegh, M. Dumont, S. Jequier, Human gastrocnemius medialis pennation angle as a function of age: from newborn to the elderly, J. Physiol. Anthropol. Appl. Hum. Sci. 20 (2001) 293–298.
[102] R.I. Kelly, R. Pearse, R.H. Bull, J.L. Leveque, J. de Rigal, P.S. Mortimer, The effects of aging on the cutaneous microvasculature, J. Am. Acad. Dermatol. 33 ((1995) 749–756.
[103] G.J. Hodges, L. Sharp, R.E. Clements, D.F. Goldspink, K.P. George, N.T. Cable, Influence of age, sex, and aerobic capacity on forearm and skin blood flow and vascular conductance, Eur. J. Appl. Physiol. 109 (2010) 1009–1015.
[104] R. Sinclair, A. Chapman, J. Magee, The lack of significant changes in scalp hair follicle density with advancing age, Br. J. Dermatol. 152 (2005) 646–649.
[105] I. Singh, A.P. Morris, Performance of transdermal therapeutic systems: Effects of biological factors, Int. J. Pharm. Investig. 1 (2011) 4–9.
[106] K.V. Roskos, H.I. Maibach, R.H. Guy, The effect of aging on percutaneous absorption in man, J. Pharmacokinet. Biopharm. 17 (1989) 617–630.
[107] M.T. Holdsworth, W.B. Forman, T.A. Killilea, K.M. Nystrom, R. Paul, S.C. Brand, R. Reynolds, Transdermal fentanyl disposition in elderly subjects, Gerontology 40 (1994) 32–37.
[108] K. Doki, A.S. Darwich, N. Patel, A. Rostami-Hodjegan, Virtual bioequivalence for achlorhydric subjects: the use of PBPK modelling to assess the formulation-dependent effect of achlorhydria, Eur. J. Pharm. Sci. 109 (2017) 111–120.